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Joint fluid leukocytosis of patients with rheumatoid arthritis Evidence for neutrophil and monocyte chemotaxis in vivo

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SUMMARY The cell picture of the synovial fluid of fourteen patients with rheumatoid arthritis was studied in smears contrasted with the May-Grünwald-Giemsa stain. The cytology was dominated by neutrophils, many with signs of necrobiosis. The mononuclear cells displayed signs of proliferation and differentiation. Comparison with the immobile erythrocyte provided evidence that the accumulation of leukocytes in the synovial fluid of patients with rheumatoid arthritis was due to active leukocyte migration, presumably stimulated random movement and chemotaxis.

Key words: Rheumatoid Arthritis, Synovial Fluid, Neutrophils, Mononuclear Cells, Chemotaxis.

INTRODUCTION

The directional movement (chemotaxis) of polymorphonuclear (PMN) and mononuclear (MN) leukocytes from peripheral blood has been studied mainly in vitro. The accumulation of leukocytes in inflammatory exudates is thought to reflect their chemotactic movement into the exudate (1,2). The idea that leukocyte chemotaxis exists in vivo has, however, been challenged (3,4,5).

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The synovial fluid from the inflamed joints of patients with rheumatoid arthritis provides a unique possibility to study the leukocytes in their function compartment (6). The synovial fluid approximates a physiological milieu, the leukocytes of which can be studied qualitatively and quantitatively.

The present study was performed to elucidate the question of leukocyte chemotaxis in vivo. The synovial cytology in patients with rheumatoid arthritis is described and compared with the corresponding cytology in the peripheral blood.

MATERIAL AND METHODS

Patients

Twenty-seven consecutive patients from the out-patient clinic of the Department of Rheumatology, Umeå, were included in the

study, as described in a previous paper (7). They had classical or definite rheumatoid factor positive arthritis as defined by the criteria of the American Rheumatism Association (8).,

Preparation of smears from the synovial fluid

Synovial fluid was collected during routine needle aspiration of knee joints. It was performed with a sterile disposable syringe and without using local anaesthetics. The first millilitres of the synovial fluid were thoroughly shaken. Then 0.1 ml of the fluid was mixed with 9.9 ml of Gey's solution in order to prevent jelling of the exudate (6). The Gey's solution used was supplied with bovine albumen (Sigma A4503), final concentration 1 % w/v, in order to make the cells stainable with the May-Grünwald-Giemsa stain. Standardized smears were obtained from 0.4 ml of the cell suspension by means of a cytocentrifuge (Shandon-Elliot Cytospin®), 1,000 r.p.m., 10 minutes (Fig. 1).

Differential counting of the smear cytology

Classification of the cells in the smears of the synovial fluid was performed blind-fold by BN (Table 1), according to conventional hematological criteria (9). The PMNs were defined as having a segmented nucleus when the breadth of the intersegmental chromatine was threadlike. Degranulated and vacuolated PMNs were regarded as vital and active. PMNs with nuclei showing signs of karyorrhexis (isolated drop-like nuclear segments) or pycnosis (one round nucleus with condensed chromatine) were designated as "pycnotic". The lymphocyte-like cells were arbitrarily divided into "small lymphocytes", i.e., smaller than the PMNs in the same visual field, and "large lymphocytes", i.e., larger than the PMNs in the same visual field. The flattening of cells during cytocentrifuge preparation made other assessment of cell size hazardous. There was a gradual transition of monocyte-like cells towards classical macrophages. Cells with cytoplasmic vacuoles were classified as macrophages (Fig. 1 b, d). Due to the mentioned difficulties of classification, all mononuclear cells were grouped in one category in the calculations and plottings. The term "mononuclear cells" (MNs) thus denotes all kinds of lymphocytes, monocytes and macrophages.

RESULTS

Out of 27 consecutive patients with rheumatoid arthritis, the staining of the cells from the synovial fluid of 14 patients approached hematologicol quality and provided a basis for qualitative and quantitative study (Figs. 1,2, Table 1). The high drop-out rate was due to imperfect staining. The cells in the drop-out smears were stained dark and dim. The specific granulation of the PMNs could not be recognized.

The evaluable smears of the cells from the synovial fluid of patients with rheumatoid arthritis were distinguished by neutrophil dominance (Fig. 1a, Table 1). No basofil PMN was found. Occasional eosinophils were recorded (1/1,500 cells counted).

The neutrophils of the joint fluid were mature with only 3/1,400 cells with staff nucleus as compared with 33/1,400 cells with staff nucleus in the corresponding differential counts from peripheral blood. There was no overlapping of these figures as estimated by 99 % confidence intervals. A considerable number of the joint neutrophils showed pycnotic changes.

It is evident from Figure 1b and from Table 1 that a number of macrophages were found in the smears of synovial fluid. This picture suggested macrophage transformation of monocytes in the synovial fluid, since macrophages are not found in smears of peripheral blood.

In two patients with rheumatoid arthritis in remission the synovial cell picture was dominated by mononuclear leukocytes. The

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ents with rheumatoid arthritis the synovial cell picture was mononuclear leukocytes. The leukocyte concentrations were low in these patients. A few mitotic figures were seen in the smears from these patients (Fig. 1d).

In most patients with rheumatoid arthritis, the picture of mononuclear cells was distinguished by a reduction of lymphocytes and an increase of monocytes and macrophages (Table 1) when compared with ordinary blood smears. Occasional plasmocytes were also seen in the joint fluid smears.

In order to make clear the leukocyte picture in the synovial fluid, the PMN concentration in the synovial fluid was plotted against the corresponding PMN concentration in the peripheral blood (Fig. 2). Likewise, the MN concentration in the synovial fluid was plotted against the corresponding MN concentration in the peripheral blood (Fig. 2). This diagram indicates that the accumulation of leukocytes in the synovial fluid was mainly due to an accumulation of PMNs.

DISCUSSION

The high drop-out rate of MGG-stained smears was not due to overstaining. Instead, the dark, dim staining of the drop-out smears resembled the metachromasia seen in bone marrow smears after anti-coagulation with heparin. It is reasonable to assume that remnants of the mucus substances in the synovial fluid had interfered with the staining.

The accumulation of neutrophil PMNs in the synovial fluid of patients with rheumatoid arthritis is an interesting phenomenon, which could be explained by the trapping of random-moving PMNs in the joint cavity or by active migration of PMNs into the synovial fluid. Active migration appears to be the more likely mechanism. This idea leads to the question as to which kind of active migration could explain the neutrophil accumulation in the synovial fluid.

In vitro studies have provided evidence that the PMN migration is composed of non-stimulated random movement, stimulated random movement, chemotaxis resistant to microtubule antagonists (MAs, e.g., colchicine), and MA-sensitive chemotaxis (10).

The known presence of complement and immunoglobulins in the synovial fluid provide the conditions of stimulated random movement or, in the presence of a concen-

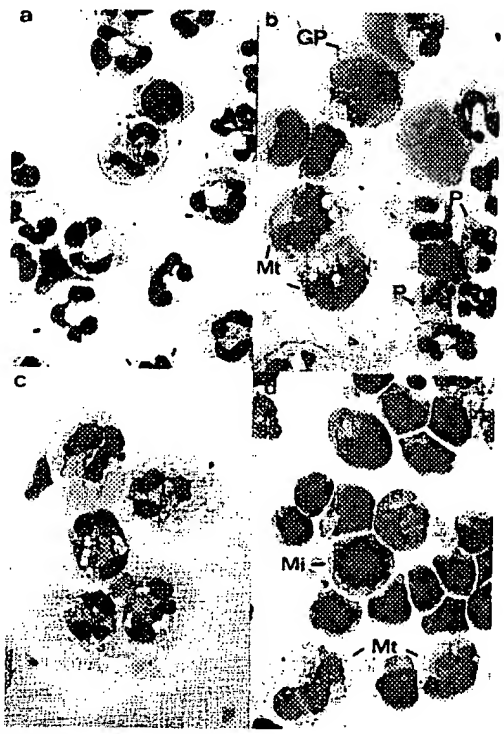

Figure 1: Cytocentrifuge-prepared smears from the synovial fluid of patients with rheumatoid arthritis. May-Grünwald-Giemsa stain. Basic magnification

a) The cell picture is dominated by neutrophil granulocytes.
b) Macrophage transformation of monocytes (Mt), dying neutrophils with caryorrhectic nuclei (P) and a macrophage (GP) which contained debris appearing to be remnants of disintegrating neutrophils.
c) Neutrophils with degranulation of parts of the cytoplasm and cytoplasmic vacuoles, presumably phagocytic.
d) The cell picture of this patient was dominated by mononucleated cells, one of which is caught in the metaphase of cell division (Mi).

tration gradient, a direct-acting MA-resistant PMN chemotaxis (11,12).

Furthermore, there is ample evidence of phagocytosis in the synovial fluid of patients with rheumatoid arthritis (Fig. 1b), i.e., a condition basic to the MA-sensitive chemotaxis (12). Dying cells are also reported to attract PMNs (13), and plenty of dying cells are found in the synovial fluid of patients with rheumatoid arthritis.

The picture of MNs in the synovial fluid was distinguished by a transformation of monocytes into macrophages with cytoplasmic vacuoles (Table 1, Fig. 1b). This picture could be explained by:

1. A selection of blood MNs capable of chemotactic movement into the synovial fluid and phagocytosis
2. A differentiation of blood MNs after their arrival into the exudate of the joint cavity (cf. Fig. 1b).
3. A proliferation of the MNs in the synovial fluid (cf. Fig. 1d).

A differentiation of blood MNs into macrophages was evident (Fig. 1b, Table 1); macrophages are not found in peripheral blood.

A proliferation of MNs in the synovial fluid was evidenced by the occurrence of mitotic figures (Fig. 1d). This proliferation appears, however, not to be of quantitative significance (Fig. 2); there was a relative dominance of MNs in only two patients (Table 1), the absolute concentration of joint leukocytes was low in these patients and these patients were approaching clinical remission. It is reasonable to assume that the cytological course of remission is distinguished by a disappearance of PMNs, followed by a disappearance of MNs.

The classical model of inflammation is that the PMNs arrive first at the site of inflammation, followed by the MNs (1,2). The cytological picture of the synovial fluid of patients with rheumatoid arthritis suggested that there is a repeated inflammatory lesion which continuously attracts PMNs into the joint. The PMNs arrive, phagocytose, degranulate, and disintegrate. The monocytes and macrophages act as scavenger cells

Table 1: *The differential count of nucleated cells in the joint exudate of 14 patients with rheumatoid arthritis. Nst: neutrophil leukocyte with staff nucleus. Nseg: neutrophil leukocyte with segmented nucleus. Npyc: neutrophil leukocyte with pycnotic or karyorrhexic nucleus. Ls: small lymphocyte. Ll: large lymphocyte. Mc: monocyte. Mf: macrophage. Pc: plasmocyte.*

Proband	Nst	Nseg	Npyc	Ls	Ll	Mc	Mf	Pc
1	-	82	-	16	-	1	1	-
2	-	52	1	19	11	2	13	2
3	-	53	16	20	2	4	5	-
4	3	79	-	11	-	2	2	3
5	-	40	-	46	6	6	2	-
6	-	77	2	14	1	2	4	-
7	-	87	-	-	-	4	9	-
8	-	94	3	2	-	1	-	-
9	-	77	-	14	-	7	2	-
10	-	90	-	-	4	-	6	-
11	-	67	6	-	9	-	8	-
12	-	-	-	60	10	4	26	-
13	-	61	-	12	11	5	11	-
14	-	91	3	1	-	-	5	-
Median	0	77	0	13	2	2	5	0
Q1-Q3	0-0	53-87	0-3	1-19	0-9	1-4	2-9	0-0
Range	0-3	0-94	0-16	0-60	0-11	0-7	0-26	0-3

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14 patients with rheumatoid arthritis.
te with segmented nucleus. Npyc:
Ls: small lymphocyte.
e. Pc: plasmocyte.

Mc	Mf	Pc
1	1	-
2	13	2
4	5	-
2	2	3
6	2	-
2	4	-
4	9	-
1	-	-
7	2	-
-	6	-
-	8	-
4	26	-
5	11	-
-	5	-
2	5	0
1-4	2-9	0-0
0-7	0-26	0-3

Fig. 2

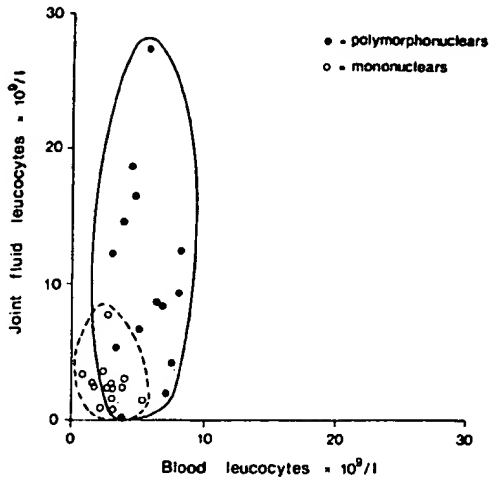

Figure 2: In fourteen patients, a differential count was made from both peripheral blood and synovial fluid (Table 1). The absolute number of joint neutrophils (•) and joint mononuclears (o) was then plotted against the corresponding values in peripheral blood. It is evident that the leukocyte accumulation in the synovial fluid was mainly due to an accumulation of neutrophils.

which phagocytose and remove debris and dead PMNs. This idea is derived from the observation of numerous dying PMNs and numerous macrophages with presumably phagoyctic vacuoles, some of which appear to contain remnants of disintegrated PMNs (Fig. 1b).

It is well known that normal synovial fluid contains very few leukocytes, usually far less than one tenth of the leukocyte concentration in peripheral blood (cf. Fig. 2). The leukocyte accumulation in the joint fluid of patients with rheumatoid arthritis could, however, be due to a passive migration with the inflammatory exudate. In this respect, comparison with the erythrocyte may be helpful. The erythrocyte is thought to lack active motility.

The normal ratio between erythrocytes and leukocytes in peripheral blood is 1000:1. It is evident from figure 1 that the number of PMNs and MNs greatly exceeded the number of erythrocytes in the synovial fluids examined, despite puncture bleeding, providing a conservative bias. This observation suggests that PMN accumulation and MN accumulation in the synovial fluid of patients with rheumatoid arthritis is due to active migration - stimulated random movement, MA-resistant chemotaxis, and MA-sensitive chemotaxis. The relative strength of each mechanism cannot be assessed at present and may vary in different patients and within the same patient in different phases of their rheumatoid disease.

It should be emphasized that the role of leukocyte migration in rheumatoid arthritis is still obscure. A clinical trial of griseofulvin blockage during one year of the MA-sensitive chemotaxis suggested that the MA-sensitive chemotaxis has a reparative role in rheumatoid arthritis (14).

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